

Adult *Gnathostoma* cf. *binucleatum* Obtained from Dogs Experimentally Infected with Larvae as an Etiological Agent in Mexican Gnathostomiasis: External Morphology

MASATAKA KOGA,^{1,6} HIROSIGE AKAHANE,² KATSUMI OGATA,³ RAFAEL LAMOTHE-ARGUMEDO,⁴ DAVID OSORIO-SARABIA,⁴ LUIS GARCÍA-PRIETO,⁴ AND JUAN MANUEL MARTÍNEZ-CRUZ⁵

¹ Department of Parasitology, Faculty of Medicine, Kyushu University, Fukuoka 812-8582, Japan,

² Department of Parasitology, School of Medicine, Fukuoka University, Fukuoka 814-0180, Japan,

³ Department of Dermatology, Miyazaki Medical College, Kiyotake, Miyazaki 889-16, Japan,

⁴ Laboratorio de Helmintología, Departamento de Zoología, Instituto de Biología, Universidad Nacional Autónoma de México 04510 D. F., México, and

⁵ Pedro García, No. 918 Tierra Blanca, Veracruz, México

ABSTRACT: We examined the muscles of 3 pelicans and obtained as many as 570 gnathostome larvae. Each of 2 dogs was experimentally infected with 20 larvae obtained using this method. Eight and 9 mo later, 4 and 9 adults were obtained from the gastric nodules in each dog, respectively. The morphology of the worms and eggs was examined, primarily using scanning electron microscopy (SEM) to identify the species. The adults demonstrated tridentate cuticular spines in their anterior forefront regions. The shape of the cuticular spines changed to di- and monodentate forms in the anterior one-third of the body. Very minute monodentate spines covered the posterior two-thirds of the body. The ventral surface of the tail of the male had 4 pairs of caudal papillae and 3 pairs of small papillae. The spines in this area were short. The morphological characteristics of the adults examined for this study were very similar to those of *Gnathostoma spinigerum*. One noticeable difference between *G. spinigerum* and the present specimens was the egg surface morphology. We found no pits on the eggshell surface of our specimens. In contrast, *G. spinigerum* has clear pits on its eggshell. The 3 previously reported gnathostomes indigenous to Latin America, *G. turgidum*, *G. procyonis*, and *G. americanum*, also have many pits on their eggshells. The adult worms of these 3 species have multidigitated spines on their anterior regions, and except for *G. turgidum*, obvious spines cover their entire body surfaces. However, the eggs of *G. turgidum* have bipolar plugs. In these latter features, the present species was more similar to *G. binucleatum*.

KEY WORDS: adult *Gnathostoma binucleatum*, gnathostomiasis, México, morphology, eggshell surface, *Pelicanus erythrorhynchos*, SEM.

The first human cases of gnathostomiasis in México were reported by Peláez and Pérez-Reyes (1970). Since 1990, the number of such patients has dramatically increased, especially in the Oaxaca-Veracruz and Sinaloa-Culiacán areas (Ogata et al., 1998). The normal source of infection for humans is considered to be the consumption of either raw or insufficiently cooked freshwater fish, mainly tilapia (Almeyda-Artigas, 1991). There have also been sporadic case reports regarding ocular and cutaneous gnathostomiasis (Hernández-Ortiz et al., 1982; Martínez-Cruz et al., 1989). Lamothe-Argumedo et al. (1989) found *Gnathostoma* sp. larvae in tilapia from the Presidente Miguel Alemán Reservoir in Temazcal, Oaxaca-Veracruz. Almeyda-Arti-

gas (1991) also found *Gnathostoma* larvae (the advanced third-stage larvae) in the muscles of fish obtained from this reservoir and named this Mexican gnathostome *G. binucleatum* (Almeyda-Artigas, 1991). Three domestic cats were experimentally infected with these larvae, and 3 female worms were later obtained from the stomach of one of them. In addition, Almeyda-Artigas (1991) also found adult worms of this species in the stomachs of a wild ocelot (*Felis pardalis pardalis*) and a stray cat.

We examined pelicans (*Pelicanus erythrorhynchos*) (a paratenic host) from the same reservoir and obtained many advanced third-stage larvae from their muscles. After experimentally infecting dogs, adult worms were also obtained from these hosts. We report the first scanning electron microscopy (SEM) profile of adult *G. binucleatum*.

⁶ Corresponding author
(e-mail: masakoga@parasite.med.kyushu-u.ac.jp).

Materials and Methods

Harvesting gnathostome larvae

We collected 3 pelicans from the Presidente Miguel Alemán Reservoir in Temazcal and examined their muscles to collect gnathostome larvae. The muscles were removed, chopped into small pieces, and the pieces cut into thin slices. The slices were then placed between 2 glass plates (10 by 10 cm), pressed by hand, and examined under a dissecting microscope. The inspected muscle remnants were next digested in artificial gastric juice (0.2 g pepsin in 0.7 ml HCl/100 ml distilled water) for 3 hr at 37°C to harvest any larvae that had been overlooked. We eventually found a total of 570 larvae. We examined pelicans instead of the intermediate fish host to collect the larvae in a short period, as it was much easier to collect a large number of larvae from pelicans than from fish. Because pelicans consume a large number of many different types of fish, gnathostome larvae accumulate in their muscles, and the nematodes are more easily collected.

Adult worm investigation

We supposed that *G. binucleatum* would have a broad range of natural mammalian hosts. Acevedo-Hernández et al. (1988) earlier reported that *Gnathostoma* eggs found in the feces of dogs and pigs in Temazcal were morphologically similar to those of *G. hispidum* (Fedtschenko, 1872) and not to those of *G. binucleatum*. However, we examined *G. hispidum* eggs from China using both ordinary optical and scanning electron microscopy and found them to be similar to those of *G. spinigerum* (Koga, 1996). We then searched for adult worms in hosts from the same district. Four opossums (1 *Philander opossum* and 3 *Didelphis marsupialis*) and 2 raccoons (*Procyon lotor*) were collected. The stomachs of these animals, however, were negative for *Gnathostoma*. In addition, 10 fecal samples from 2 pigs and 8 dogs were also examined for *Gnathostoma* eggs, but none were found. Thus, the range of natural final hosts is not as broad as we expected.

Experimental infection

Two dogs were each infected experimentally with 20 larvae obtained from pelicans, and thereafter, they were maintained at an animal center. To assess egg shedding, fecal examinations were performed once a month starting at 5 mo postinfection. Gnathostome eggs were first observed in the feces 8 and 9 mo after infection. The dogs were then anesthetized by sodium barbital and killed by bleeding from the cervical arteries. The peritonea were then gently opened, and the stomachs were removed and opened by cutting along the lesser curvature. A single hard nodule was evident in the mucous membrane in each stomach. In the nodules from each dog, 4 (1 ♂, 3 ♀) and 9 (6 ♂, 3 ♀) adult worms were found. After optical observation, the adult worms were processed for SEM. The eggs were removed from the uteri of the gravid female worms for further examination. One pair of adult worms consisting of 1 male and 1 female worm from each dog was deposited at the Meguro Parasitological Museum in

Tokyo as representative voucher specimens (Accession No. 19726).

SEM sample preparation

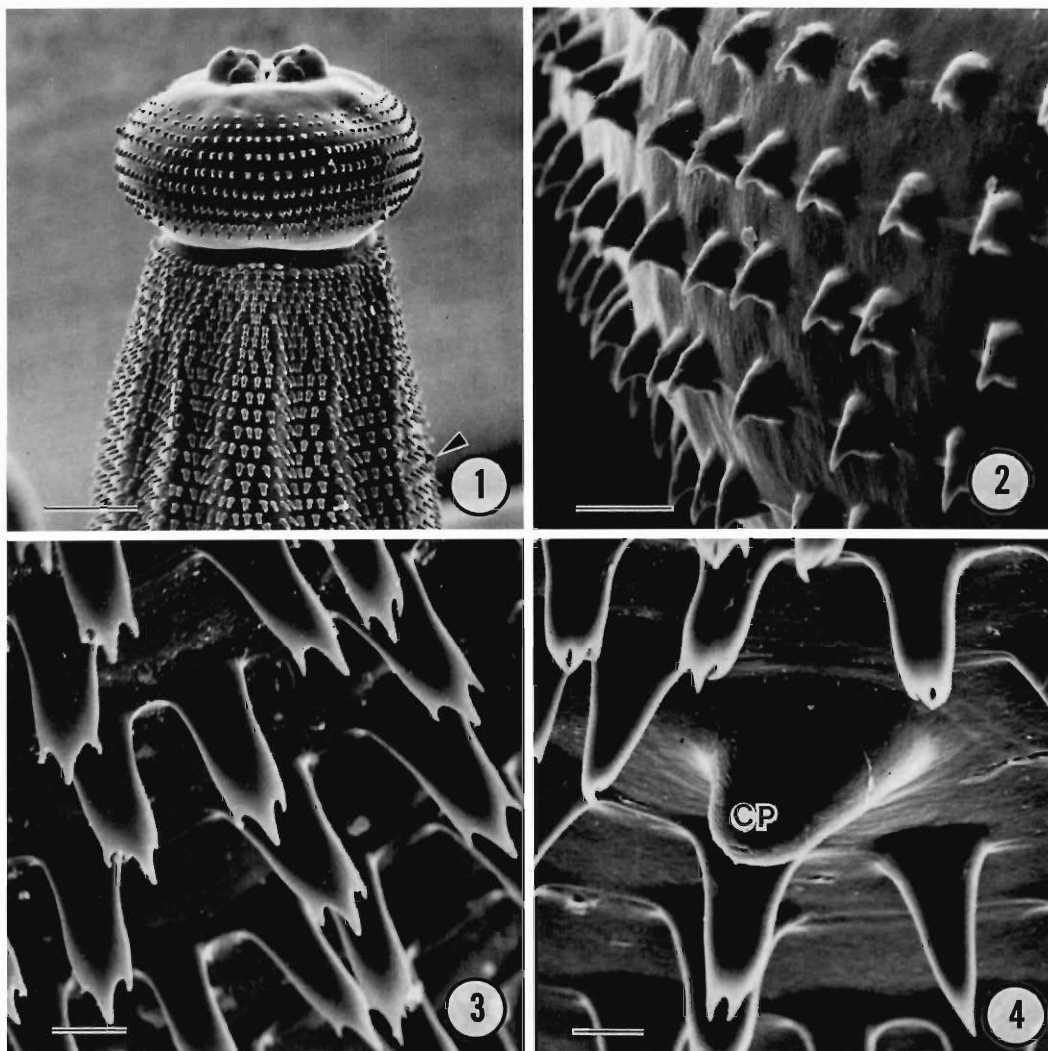
Viable adult male and female worms were washed in several changes of tap water and soaked in physiological saline solution. The worms were fixed in 10% formalin for at least 1 wk, washed in running tap water overnight to remove the fixative, then transferred to distilled water. The specimens were rinsed twice in Millonig's phosphate buffer and postfixed for 3 hr in 1% OsO₄ in the same buffer. During postfixation, the worms were cut transversely into 7 pieces to facilitate observations by SEM. These pieces were dehydrated in an ascending ethanol series, transferred into amyl acetate, and critical-point dried with a Hitachi HCP-2 critical-point dryer. The specimens were sputter coated with gold and examined with a JEOL JSM-U3 SEM operated at 15 kV.

Results

The adult male and female specimens of our *Gnathostoma* sp. had a hemispherical head bulb armed with 8–9 transverse rows of cephalic hooks (Fig. 1). The hooks had tapering points composed of hard keratin (about 7–8 μm in length) that emerged from a conical chitinous base (Fig. 2). The body spines immediately behind the cephalic bulb were tridentate (Fig. 3) and not multidigitated. One pair of cervical papillae was located laterally near the twentieth transverse striation (Fig. 1, arrow) and had a mammiform shape. The shapes of the spines around these papillae were mixed, with 2–3 denticles, and only rare unidentate spines (Fig. 4). A domelike excretory pore was situated ventrally a little behind the cervical papillae and was covered only by single denticle spines (Fig. 5). These unidentate spines gradually decreased in size posteriorly along the body (Fig. 6), and most posterior spines were minute (Fig. 7).

On the ventral side of the tail of the male, single-toothed unidentate spines were densely distributed over the entire extremity. Four pairs of caudal papillae (a, b, c, and d) and a few small papillae, which bore no spines (arrows), were also seen on this side (Fig. 8).

The fertilized uterine eggs of this *Gnathostoma* sp. were oval (66 ± 2.92 by 40 ± 3.1 μm) and had an operculum (Fig. 9, OP) on one end. The eggshell surface was plain and without pits (Fig. 10). This plain, nonpitted appearance is characteristic of this species and is the major feature differentiating *G. spinigerum* from other gnathostome species.

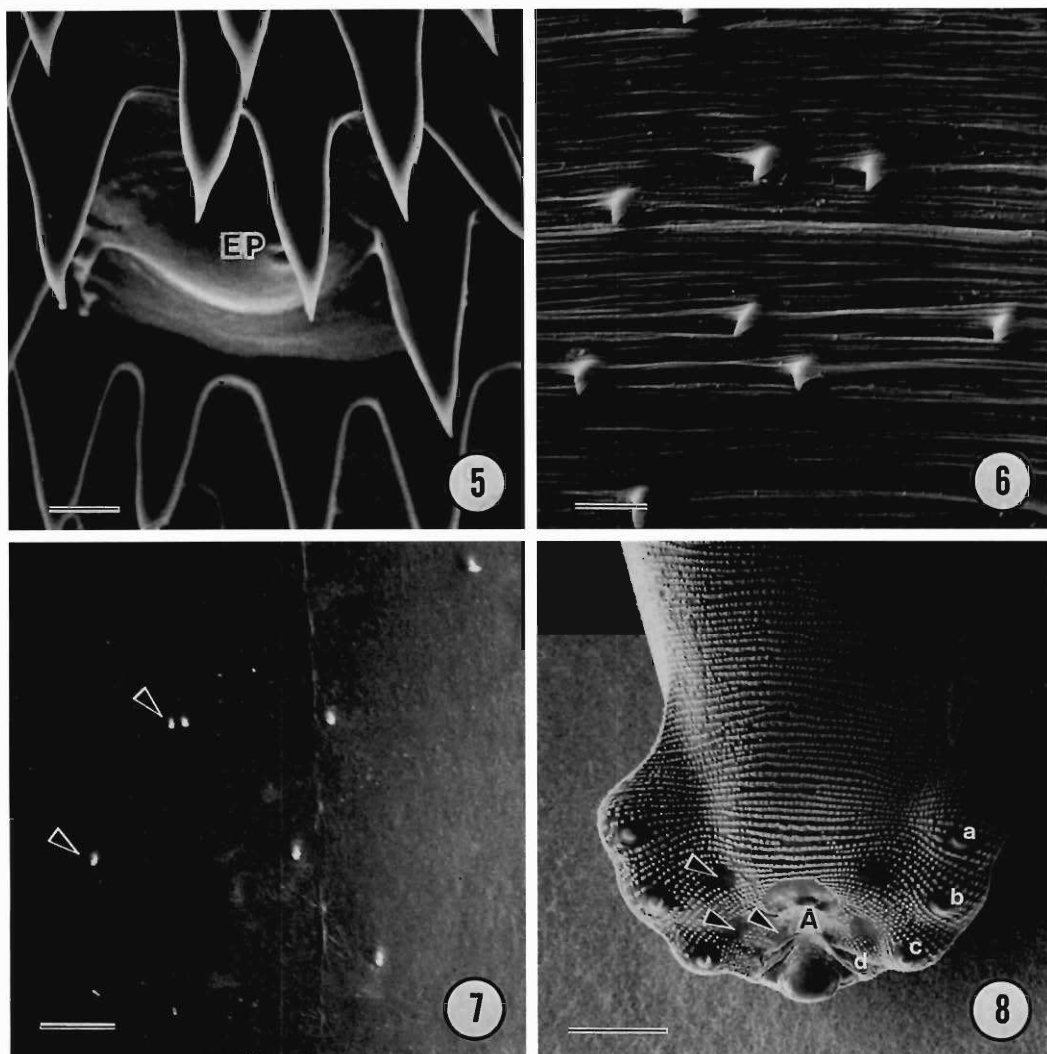


Figures 1–4. 1. Lateral view of the head bulb of *Gnathostoma* sp. The arrow indicates cervical papilla. Bar = 100 μ m. 2. A higher magnification of the hooks on the head bulb, which have conical bases and acute tips. Bar = 10 μ m. 3. Short, stumpy tridentate spines lying immediately behind the head bulb. Bar = 10 μ m. 4. Mammiform cervical papilla (CP) located between the eighteenth and twentieth transverse striations, with spines on the body surface. Bar = 10 μ m.

Discussion

Almeyda-Artigas (1991) experimentally obtained female worms from a cat stomach that could not produce fertilized eggs in the host feces. Therefore, his description of the eggs was limited to eggs from naturally infected mammals. The eggs were 64 by 38 μ m in size, with a plug at one end. The size of the eggs was similar to that of our specimens. Almeyda-Artigas (1991) considered these worms to be a new species and named them *G. binucleatum* in ref-

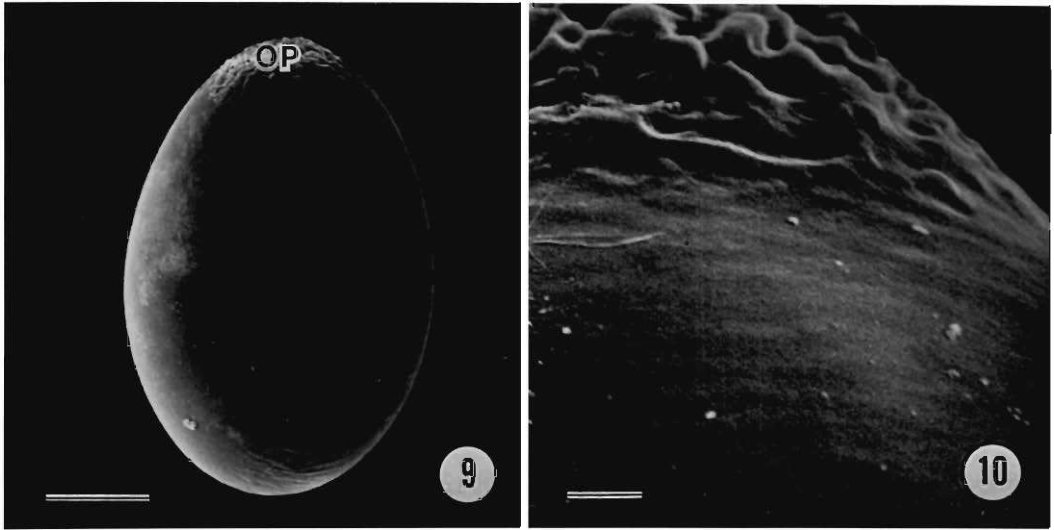
erence to the presence of 2 nuclei in the intestinal cells of the advanced third-stage larvae. Based on this general description of the morphology of the surface spines of adult worms and of the ventral side of the tail of the male by light microscopy, our belief is that the worms described by Almeyda-Artigas (1991) were very similar to *G. spinigerum*. However, the morphological details were not described precisely enough for us to be certain of this similarity. Akahane et al. (1994) reported that most of the



Figures 5–8. 5. Dome-shaped excretory pore (EP). Around this pore, all spines are unidentate with acute tips. Bar = 10 μ m. 6. Smaller unidentate spines located on posterior one-third of body. Bar = 10 μ m. 7. Very minute spines (arrows) are sparsely distributed around the terminal area of the body. Bar = 10 μ m. 8. Ventral surface of the tail of the male. Four pairs of dome-shaped caudal papillae (a, b, c, and d) and several small flat papillae without spines (arrows) are evident. Dense minute cuticular spines oriented anteriorly are also evident. A = anal pore. Bar = 20 μ m.

larvae from Temazcal that they examined had 4 nuclei in the intestinal cells. Moreover, the other features of those advanced third-stage larvae were very similar to those of *G. spinigerum*, except for the number of hooklets in each row of the head bulb. Koga et al. (1991) reported the surface ultrastructure of both adults and eggs of *G. spinigerum* from Thailand using SEM. By comparison, in the adult *G. spinigerum* from

Thailand, the spines immediately behind the cephalic bulb were broad, blunt, and multidigitate in *G. spinigerum*, while the corresponding spines of the Mexican specimens were more slender and only tridentate. The spines around the excretory pore are tridentate in *G. spinigerum*, but the corresponding spines of the Mexican specimens are unidentate. All specimens in this study had minute cuticular spines, even on the



Figures 9, 10. Egg from the uterus of a gravid female of *Gnathostoma* sp. from Mexico having an operculum on one end, showing smooth surface. OP = operculum. Bars = 20 and 1 μ m, respectively.

posterior half of the body. However, *G. spinigerum* is usually reported to have a naked posterior half (Miyazaki, 1960). The ventral appearance of the tail of the male was almost the same in our specimens as that of *G. spinigerum*. The most noticeable difference between *G. spinigerum* and the Mexican species is therefore the surface of the eggshell. The eggshells of *G. spinigerum* and other gnathostome species have many pits on the surface, and the shape of these pits is species-specific (Koga, 1996), while the eggshells of the Mexican species are not pitted.

In Central and South America, 3 species of gnathostomes have been recorded: *G. procyonis* (Chandler, 1942) from a raccoon in Texas, *G. turgidum* (Stossich, 1902, quoted in Travassos, 1925) from an opossum, and *G. americanum* (Travassos, 1925) from *Felis tigrina*, the latter two in Brazil. The adults of these 3 species have multidigitate (4–5 teeth) spines on their anterior regions and dense spines on the posterior half of their body surfaces, except for *G. turgidum*. In contrast, our specimens had very minute spines sparsely distributed over the posterior half of their bodies, and these spines were recognizable only by SEM examination. In *G. procyonis*, the eggs have a plug on either pole and also have many pits on the eggshell surface. The eggs of *G. turgidum* and *G. americanum* have bipolar plugs on both sides and thus can be easily dis-

tinguished from those of *G. spinigerum*. Our Mexican specimens had 1 operculum at one end and no pits on the surface. Both the adults and larvae of the present species were very similar to those described as *G. binucleatum* by Almeyda-Artigas (1991).

We therefore tentatively consider our specimens to be *G. binucleatum*, but note that the taxonomic status remains inconclusive. We consider it premature at this point to propose a new species, pending further studies. The description by Almeyda-Artigas (1991) does not provide sufficient information for a definitive determination of the status of our specimens; thus, we must tentatively assign them to *G. binucleatum*. Further study of both species is needed to determine the specific status of each and to evaluate *G. binucleatum* in relation to the characters described in this study.

In Latin America, human gnathostomiasis has previously been reported in Ecuador (Ollague-Loaiza et al., 1981). It is highly possible that migratory waterfowl may have spread the same species of nematode to various Latin American countries. It is possible that the Ecuadorian parasite might be the same species as in the present study, because in Ecuador adult worms were also recovered from domestic cats and dogs infected both naturally and experimentally (Ollague-Loaiza et al., 1985, 1987; Ollague-Tor-

res and Buchelli de Cevallos, 1985). The morphology of those worms was also quite similar to that of our Mexican specimens. The Ecuadorian specimen was identified as *G. spinigerum*, but this designation is uncertain, and specimens from that area should be reexamined.

Acknowledgments

The authors are greatly indebted to Agustín Jiménez Ruiz and Martín García Varela, Laboratory of Helminthology, Institute of Biology, Autonomous National University of Mexico, for their valuable assistance. We gratefully acknowledge the valuable help of Professor I. Tada, Department of Parasitology, Faculty of Medicine, Kyushu University, for his critical reading of earlier drafts. We also thank Dr. K. Kawashima, Professor Emeritus, Kyushu University, for his advice on the zoological nomenclature. Our sincere gratitude is also due to Dr. Scott Monks, The University of Toronto, for his final revision of this manuscript. This work was supported by a grant-in-aid for International Scientific Research (Field Research 08041187) from the Ministry of Education, Science, Sport, and Culture, Japan.

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